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14. ABSTRACT This project offers an exploration of a new line of diagnostics for prostate cancer. It is based on the expression of tNASP protein in prostate cancer cells. We propose to detect anti-tNASP antibodies in the serum of prostate cancer patients for screening purposes and to distinguish prostate cancer from BPH. Detection of tNASP protein expression levels in needle biopsies and postoperative prostate cancer samples will be correlated with pathologic indicators of aggressive prostate cancer for diagnostic and prognostic purposes.
We propose two specific aims:
Specific aim #1 will validate the detection of anti-tNASP antibodies as *serum-based* biomarkers of prostate cancer. This aim will address the hypothesis that tNASP-specific antibodies are present in the blood of prostate cancer patients, but not BPH patients or otherwise healthy men. We will test the hypothesis that presence of tNASP-specific antibodies is an early marker of prostate cancer and that, together with PSA assay, it could be used as a screening method for early detection in high-risk population groups.
Specific aim #2 will validate the utility of tNASP protein as a *tissue-based* diagnostic and prognostic biomarker of prostate cancer. We will address the hypothesis that expression of tNASP in prostate tissue samples obtained during needle biopsies can be used to diagnose prostate cancer and to differentiate between BPH and malignant tumor. The second part of this aim will test the hypothesis that advanced and aggressive prostate cancers show higher expression of tNASP protein than early stage tumors.

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Introduction

NASP is a histone chaperone and a facilitator of chromatin assembly that is present in all dividing cells. NASP has two splice variants: tNASP, which is highly expressed only in cancer cells, embryonic cells, and spermatocytes, and sNASP, which is found in all dividing cells (Richardson, et al., 2000). Different cancer types as well as different stages of the same cancer demonstrate specific tNASP RNA expression profiles. For example, its expression levels are elevated in grade 1 and 2 versus grade 3 in breast cancer, estrogen receptor positive versus negative tumor types, and sporadic versus BRCA1/BRCA2 mutation positive tumors (van't Veer, et al., 2002). We hypothesize that in prostate cancer tNASP expression begins to elevate at the onset of the disease and then increases further as the cancer progresses. Therefore, tNASP expression status could be used as a diagnostic and prognostic marker for prostate cancer. Over the last decade, dozens if not hundreds of proteins have been implicated in prostate carcinogenesis (Hermani, et al., 2005). Many of them have been proposed as potential tumor markers, but nevertheless did not progress to become useful clinical diagnostic tools. We believe that tNASP is a particularly strong tumor marker candidate for the following reasons. First: tNASP is an extremely autoantigenic protein (Batova, et al., 2000), and while in cancer-free patients tNASP is sequestered in an immunologically-privileged compartment behind the blood-testis barrier, high and aberrant expression of tNASP protein in prostate cancer tissues induces the development of a robust humoral immune response, which could be easily detected using anti-tNASP specific antibodies in ELISA. Second: the tNASP splice variant is a strong proliferation marker without exceptions highly expressed in number of examined cancer tissues (Ali-Fehmi, et al., 2010). Comparing to other candidates for prostate cancer markers which due to their nature could only be detected in frozen sections, tNASP is a stable protein easily detectable in frozen and paraffin sections with no cross-reactivity to other proteins.

Body

Based on the hypotheses we propose two specific aims.
Objectives: Specific aim #1 will validate the detection of anti-tNASP antibody as a serum-based biomarker of prostate cancer. This aim will address the hypothesis that tNASP-specific antibodies are present in blood of prostate cancer patients, but not in BPH patients or in otherwise healthy men. We will test the hypothesis that presence of tNASP-specific antibodies is an early marker of prostate cancer and, together with PSA assay, could be used as a screening method for early detection in high-risk population groups. Specific aim #2 will validate the applicability of detection of tNASP protein as a diagnostic and prognostic biomarker of prostate cancer in needle biopsies and postoperative tissues. We will check the hypothesis that expression of tNASP in prostate tissue samples obtained during needle biopsies can be used for diagnostic of prostate cancer and differentiation between benign BPH and malignant tumor. The second part of this aim will test the hypothesis that advanced and more aggressive prostate cancers show higher expression of tNASP protein comparing to the earlier stage tumors and, therefore, tNASP expression profile is the prognostic marker for prostate cancer.
Methods: Prostate tissue samples and sera from prostate cancer patients, BPH patients , and healthy men will be obtained from the prostate tissue facilities though collaborative arrangements. Slides will be immunostained with anti-tNASP antibody, blindly assessed, and analyzed to establish correlations with clinical and pathology diagnosis, including serum PSA, tumor volume, Gleason score, seminal vesicle involvement, and presence of metastases. Serum will be tested for the presence of anti-tNASP antibodies using ELISA with immobilized protein fragment specific for human tNASP.

Key Research Accomplishments

I. To validate the detection of anti-tNASP antibodies as serum-based biomarkers of prostate cancer I generated a series of plasmids for expression of tNASP specific protein fragment:

1. Two primers were designed
Forward primer: GAAGCAAGGGAAGAGTTGAGA
Reverse primer: TTCTTCACCCTCTTTCATCTG

Using Human Testis cDNA as a template and Taq Polymerase the PCR reaction was ran. The fragment of correct size was received (fig. 1):

Fig. 1

The PCR product was cloned in the pEXP5-CT/TOPO vector and propagated in One Shot Top10 competent cells. Two positive clones were obtained. Clone #8 was proved to have the correct orientation and sequence (fig.2)

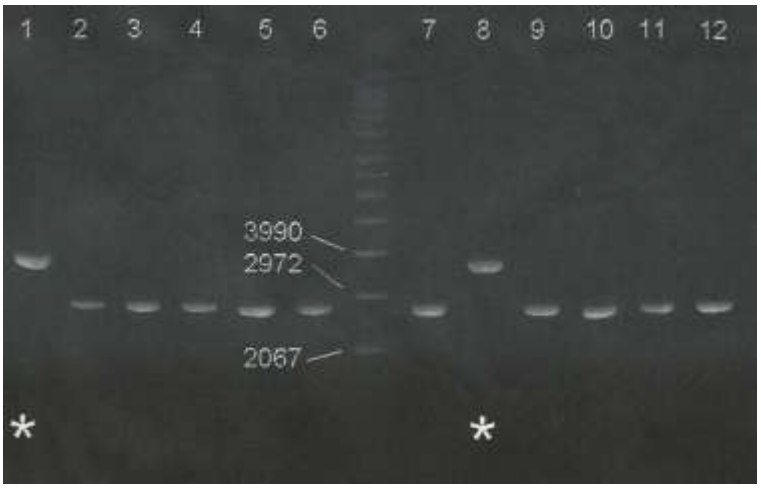

Fig. 2

The tNASP specific fragment was expressed in BL 21 AI cells and purified using NiNTA slurry (fig.3)

Fig. 3 (1, 2)

Quality of expressed protein was proved by probing with specific anti t-NASP antibody (1) and anti 5-HIS antibody (2)

II. Procured samples of sera and biopsy tissues from prostate cancer patients, benign prostate hyperplasia patients, and patients with unaffected prostate.
I used the next sources of sera and biopsies:
University of North Carolina at Chapel Hill Tissue procurement facility. Total number of samples procured from this facility is 11, control sera – 12.

III. Tested procured sera by ELISA with immobilized tNASP specific protein fragment. Sera were precipitated with ammonium sulfate to purify IgG. tNASP specific fragment

was bound to the wells of High affinity ELISA plates (Immulon-4-HBx plates). To each well 150 ng of tNASP specific fragment was bound and incubated with different dilutions of patients' serum.

On the figure 3 the reaction intensity demonstrates the amount of tNASP specific antibodies in the patients serum Colum #12 is the control serum (healthy patients to the best of our knowledge). As we can see in at least five patients serum contained significantly increased levels of specific anti-tNASP antibody (patients 3, 4, 5,7, and 9). This experiment was repeated at least three times.

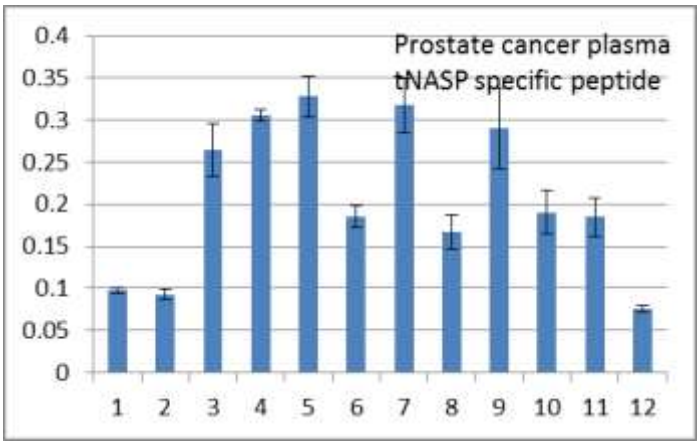

Fig. 3

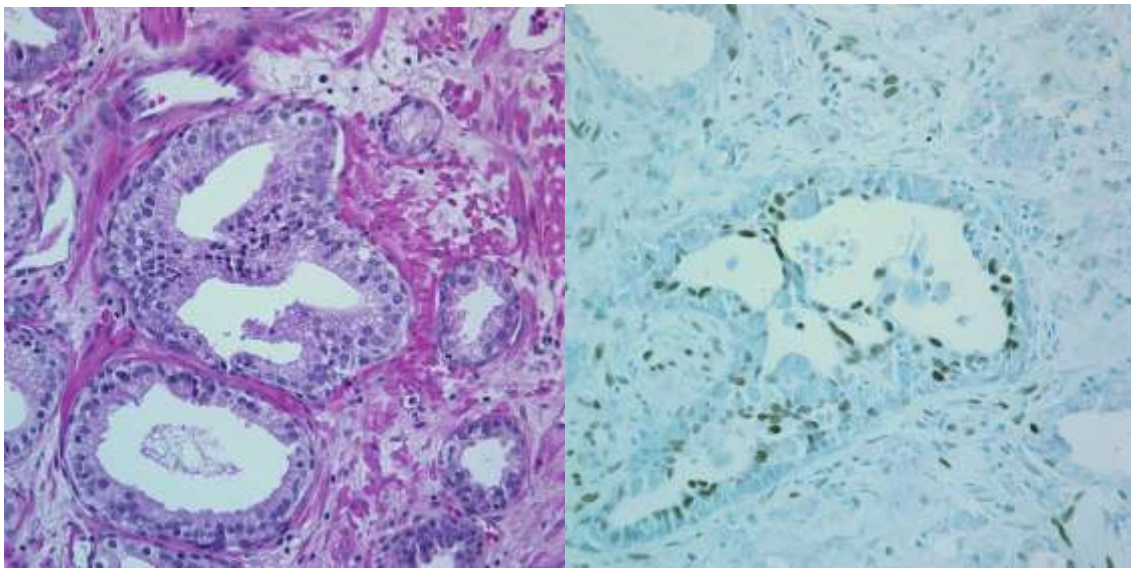

Fig.4.

Analysis of expression of tNASP protein in the prostate cancer biopsies shows that the patients with high titer of anti-tNASP antibody tend to have high level of expression of tNASP in the tissues. Left image shows Hematoxillin and Eosin staining of prostate tissue and the right image shows close fragment from the same piece of tissue stained with anti-tNASP antibody. Dark places indicate accumulation of the tNASP protein in the cells.

In the collaboration with the ROSWELL PARK CANCER INSTITUTE we received mikro biopsies for prostate cancer androgen independent (total number of samples - 36), prostate cancer androgen dependent (total number of samples - 36) and benign prostate hyperplasia samples (total number of samples - 36). The obtained samples were stained with hematoxillin-eosis, anti-tNASP antibody and control serum (preimmune serum). The preliminary results showed that highest level of expression of tNASP is in androgen-independent prostate cancer tissue, followed by androgen dependant biopsies and benign prostate hyperplasia.

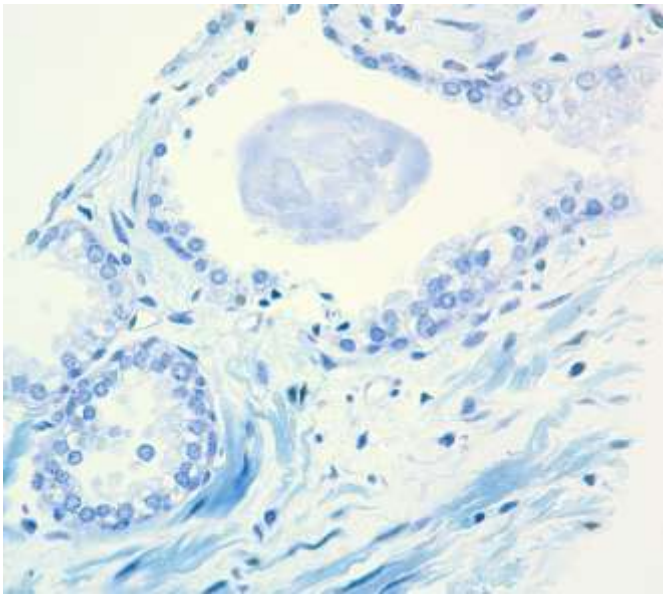

Fig. 5.

Samples from Benign Prostate Hyperplasia patients did not show any tNASP expression (fig.5) contrary to the androgen dependent and androgen independent tumors. Based on these preliminary data it looks possible to differentiate between malicious and benign tumor based on the cellular expression of tNASP.

Reportable Outcomes

Since I have changed the employment in July 2013 and about two months of active research time in the University of North Carolina at Chapel Hill were not productively used, this project is not completed yet. In my new place of employment (Campbell University) I am provided with the lab space and appropriate resources to complete this undoubtedly fascinating project. All data from serum detection of tNASP antibody present in the blood of prostate cancer patients with appropriate control samples are ready for profound correlative analysis. Biopsy samples are already cut and stained and are also ready for study and more detailed analysis.

Once the pressure from the initial teaching duties in my new employment will ease up I plan to complete the analysis of received data in summer-fall 2014. For my opinion the preliminary results are very promising and will get their further development in presentation on research conference and publication in the peer-reviewed journal.
I would love to thank you Department of Defense for providing me with this wonderful opportunity to test the hypothesis weather tNASP expression can be used as a prognostic and diagnostic tool for early detection of prostate cancer.

Conclusion

In the result of the preliminary analysis of received data we showed the following:

1. Prostate epithelial cells have significantly higher level of expression of tNASP comparing to normal prostate tissue and samples of Benign Prostatic Hyperplasia.

2. There is possible correlation between androgen-dependent and androgen-independent prostate cancer cells in the level of tNASP expression. Appropriate development of these findings could be an important prognostic test for the prostate cancer

3. It was demonstrated that the expression of tNASP in prostate generates the production of specific anti-tNASP antibody

4. Specific anti-tNASP antibody were detected in this study by ELISA with recombinant tNASP specific peptide. It can lead to the development of supplementary to PSA test for prostate cancer.

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